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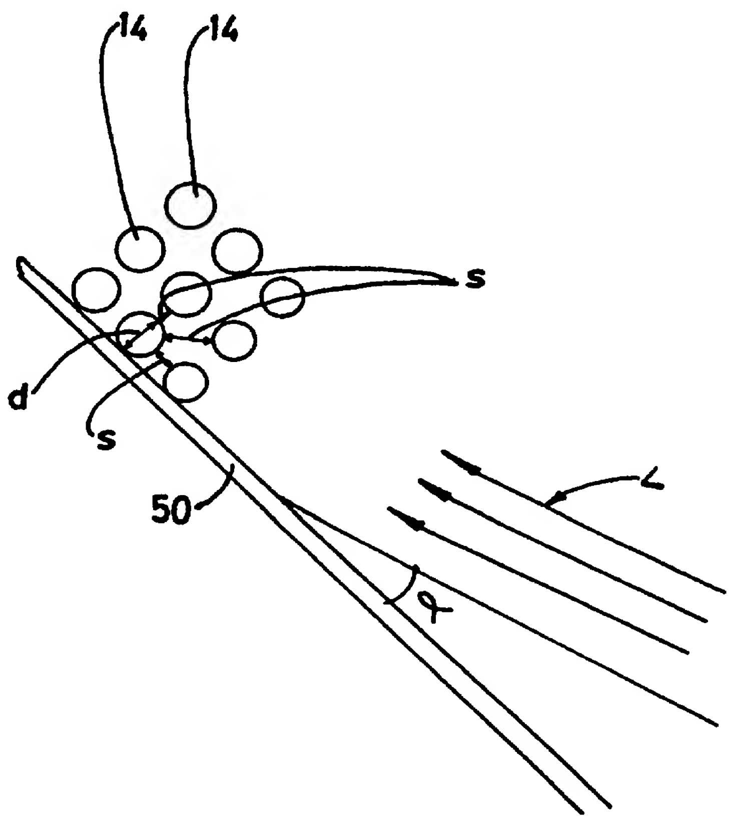
(54) Title: COLLECTIVE RESONANCES IN TWO-DIMENSIONAL ARRAYS FOR COLLOIDAL METAL FILMS

(57) Abstract

The present invention provides strong nonradiative coupling of plasmon resonances of closely spaced, metal particles arranged in semi-regular, two-dimensional arrays disposed in a colloidal metal film. The colloidal films are formed on a substrate having functional groups with high affinity to an appropriate metal, such as silver. The substrates are exposed to a colloidal suspension of metal particles having average diameters in the range of 200-1000 Ångstroms for a desired time period, resulting in the adsorption of the metal to the substrate. The electrostatic interaction between individual particles causes a semi-regular two-dimensional array to be produced. Preferably, the means distance between particles is approximately equal to, or less than, their average diameter. The present invention may be utilized as an artificial antenna, for various different types of devices utilizing the energy of light. The present invention may also be used to perform surface-enhanced spectroscopies. The present invention may further be used in biomedical applications, such as homogenous immunoassays, analysis of whole blood, and DNA probes. The present invention has enabled work on combining of polymerase chain reaction (PCR), with detection of a DNA fragment of interest (DNA probe). The PCR and DNA probe are combined in a vial, with the present invention obviating the need for any intermediate steps, to greatly facilitate DNA probe techniques.



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COLLECTIVE RESONANCES IN TWO-DIMENSIONAL ARRAYS FOR COLLOIDAL METAL FILMS

FIELD OF THE INVENTION

This invention relates to plasmon resonances, and more particularly, to collective resonances in two-dimensional arrays of silver metal particles disposed in a medium.

BACKGROUND OF THE INVENTION

Plasmon resonances are collective oscillations of electron density in metals. In order to optically excite plasmon, it is necessary to match the frequency and wave vector of incident photons simultaneously to the frequency and wave vector of the plasmon. This cannot be accomplished for smooth metal surfaces.

Colloidal suspensions of nanometer sized silver particles are a bright yellow-greenish color due to the intense bands around 400-500 nm in their extinction spectra. These bands correspond to the excitation of surface plasmon resonances as disclosed by M. Kerker, in *The Scattering of Light and Other Electromagnetic Radiation*, Academic Press, New York 1969.

The bulk plasmon frequency of a metal is that frequency at which the real part of the complex dielectric function ($\epsilon_r(\omega)$) is equal to zero. In contrast, the surface plasmon frequency of a spherical particle is determined by $\epsilon_r(\omega) = -2$, as result of fulfilling the boundary conditions. This was discussed by T. L. Ferrell, T. A. Callcott, and R. J. Warmack in *American Scientist*, Vol. 73, pp. 344-353, 1985. The frequency of the surface plasmon also depends on the size of a sphere and the dielectric environment.

Based on a phenomological description by G. Mie in *Ann. d. Physik*, Vol. 25, page 377, 1908, the multipolar charge

oscillations in a sphere can be presented as a series of spherical harmonics of different orders, as disclosed by M. Born and E. Wolf in *Principles of Optics*, Pergamon Press, New York 1993.

It is well known that oscillating molecular dipoles can couple to each other and exhibit nonradiative energy transfer when spaced at distances up to one hundred angstroms (100 Å). At long distances, that is distances greater than 100 Å, strong interaction between dipoles is not required, but partial overlap between the emission bands is necessary due to Förster mechanism. For closely spaced dipoles, that is dipoles that have a distance less than, or equal to, ten angstroms (10 Å) between them, when electrostatic interaction between the dipoles is strong, an exchange interaction can also contribute to energy transfer. This was discussed by B. Valeur, D. M. Jameson, and G. D. Reihart, Eds. in *Fluorescent Biomolecules*, pp. 271-275, New York 1989. The energy transfer is accompanied by significant changes in the absorption/emission spectra of the molecules.

The concept of interaction of plasmon resonances between particles was first introduced by S. Yamaguchi in *J. Phys. Soc. Japan*, Vol. 15, pp. 1577-1581, 1960, and the theory was developed in subsequent publications, such as a publication by T. Yamaguchi, S. Yoshida, and A. Kinbara in *Thin Solid Films*, Vol. 18, pp. 63-67, 1973, to explain the extinction spectra of silver island films. A. Wokaun in *Solid State Physics*, Vol. 38, pp. 223-294, 1984, using an approach similar to that disclosed by the above references, calculated the Raman enhancement for molecules near the surface of silver particle arrays produced by a lithographic technique.

These researchers used the local field concept in their calculations, wherein the field experienced by an individual

particle is the sum of the field of the incident radiation and fields produced by the other particles. The plasmon resonance is approximated as a dipole and interaction between dipoles is considered in the far field regime. Retardation effects are also included.

In essence, their calculations predict a shift to the red spectral region and broadening of the plasmon resonance when dipole-dipole interactions are included. J. Gersten and A. Nitzan in *Surface Science*, Vol. 158, pp. 165-189, 1985, have considered the effect of the close proximity of two (2) metal spheres on the plasmon resonance. In their calculations, the electrostatic interaction of two (2) dipoles was taken into account. As a result, the resonance frequency of this system was shifted to the red spectral region, relative to that of an isolated sphere.

However, the prior art does not disclose strong nonradiative coupling of plasmon resonances in semi-regular arrays of nanometer sized silver metal particles disposed in a medium.

OBJECTS OF THE INVENTION

Therefore, it is an object of the present invention to provide strong nonradiative coupling of plasmon resonances in semi-regular arrays of silver particles.

It is a further object of the present invention to provide a new approach to energy transfer and storage systems for the performing of immunoassays and DNA probe analyses; and for the preparation of optical devices and substrates for surface-enhanced spectroscopies that has fundamental and practical importance in various appropriate applications.

It is yet still a further object of the present invention to provide colloidal metal films of semi-regular arrays of

metallic particles which may be applied in various energy transfer and storage systems, bioanalytical procedures, and optical filters.

SUMMARY OF THE INVENTION

These and other objects are accomplished by the present invention which provides strong nonradiative coupling of plasmon resonances of closely spaced, nanometer sized, metal particles arranged in regular and semi-regular, two-dimensional arrays. The preferred embodiments of the present invention introduces these colloidal metal films (CMFs) for use as substrates for enhanced spectroscopies; optical filters; and active elements in energy-harvesting, transformation, and transfer systems.

The present invention recognizes that a matching of frequency and wave vector between incident photons and plasmon can be achieved with roughened metallic surfaces or small metal particles, such as silver particles, disposed in a medium, such as colloidal metal suspensions and island films for example.

For spheres that have a size comparable to the wavelength of light, retardation effects play an important role in determining the frequencies of the surface plasmons. The retardation effects result from differences in the phase of the incident light within a single particle, and as a consequence, the surface plasmon, or surface-charge oscillations, has multipolar characteristics.

As the size of the metal particle increases, the contribution of the higher harmonics becomes increasingly important.

For closely spaced metal particles which exhibit

plasmon resonances, coupling of the oscillating multipoles will also occur. For strong coupling, changes in the extinction spectrum of an ensemble of closely spaced particles should be observed relative to that of a system in which the particles are far apart. In addition, if the particles are arranged in a regular array, coherent effects may be present in the excitation, as well as in the decay of the plasmon, through scattering.

The colloidal metal films of the preferred embodiment of the present invention are prepared by adsorption, such as self-assembly, of metal particles on derivatized substrates. In the preferred embodiment of the present invention, the colloidal metal films are prepared on glass slides derivatized with 3-mercaptopropyl trimethoxy silane (MPS). However, the colloidal metal films can be formed on any solid substrate, that have functional groups with high affinity to silver, or other appropriate metals. For silver, such groups include -SH, -CN, pyridyl, and $-NH_2$.

In the preferred embodiment, the slides are exposed to a colloidal suspension of silver metal particles having average diameters of approximately one thousand angstroms (1000 Å) for a period of time, resulting in the irreversible adsorption of the metal to the surface, due to the formation of silver-sulphur bonds. Because of the electrostatic interaction between individual particles, a semi-regular two-dimensional array is produced. After exposure of the slides for several days to colloidal suspensions, highly dense films are formed, in which the mean distance between particles is approximately equal to, or less than, their average diameter. Alternatively, other suitable nanometer-sized metal or semiconductor particles that also have appropriate physical properties in two-dimensional semi-regular arrays may be used in the present invention.

Because interparticle spacing in a colloidal suspension of optical density 1.0 is approximately 5 μm, the extinction spectrum thereof represents the plasmon resonances of noninteracting single particles. Several distinct features are observed in the extinction spectrum of noninteracting particles, the spectrum has a maxima near 510 nanometers (nm) and a maxima near 425 nm, and two (2) shoulders, one around 380 nm and another shoulder at approximately 350 nm.

These features can be attributed to excitation of different harmonics of the plasmon resonance, for a nearly spherical shaped silver particle. The maxima at 510 nm and 425 nm in the extinction spectrum can be attributed to dipole and quadrupole harmonics, respectively, whereas the shoulders at 380 nm and 350 nm in the spectrum are due to excitation of higher harmonics.

In the preferred embodiment of the present invention, as the density of the particles in the CMF increases, a dramatic change is observed in the extinction spectrum. The lowest energy maximum shifts to the blue spectral region and collapses into a single sharp maximum peak at about 431 nm. This is an "enhanced" peak, which corresponds to quadrupole harmonics of single particles. The enhanced peak occurs when the mean distance between particles is on the order of their diameter.

Light can excite dipolar and multipolar harmonics of the plasmon resonances in the single particles. These excited harmonics will be coupled with harmonics of the nearby particles. The present invention recognizes that simple symmetry considerations from having a two-dimensional arrangement of the metal particles can result in excitation of multipolar harmonics. This conclusion is supported by the fact that the sharp enhanced peak in the extinction spectrum

is more intense in CMFs with large silver particles, which maintain an interparticle distance equivalent to their diameters.

The dependence of the extinction spectrum of a CMF, that has interparticle spacing approximately equal to the average diameter of particles therein, on the tilt angle of the film to light exposed thereto, is evidence of collective plasmon resonances of the particles. The present invention recognizes that there is no unusual angular dependence of the extinction spectra with low particle density CMFs.

However, when s-polarized light is applied to the CMF, the extinction band broadens and decreases in intensity as a tilt angle of the CMF relative to the light is increased. The extinction band broadens and decreases in intensity due to dephasing of the plasmon oscillation in neighboring silver particles. Dephasing results when the incident wavefront of the light reaching the silver particles in the colloidal film is at different phases. This results from the tilt angle increasing from a normal incident angle of zero degrees (0°) relative to the light, to a substantial angle.

The present invention further recognizes that when the CMF is exposed to p-polarized light, the dependence of the extinction spectrum is more complex. As the tilt angle of the CMF is increased from the normal incident angle of 0°, the enhanced sharp peak or main maximum in the spectrum, at about 431 nm, first decreases in intensity, slightly broadens, then shifts to the red spectral region. The plasmon oscillation reaches a minimum in intensity at around a 30° angle of the film relative to the light. The maximum continues to shift to the red spectral region, increases in intensity, and reaches another maximum around 55° of the film

to the light at the 471 nm position of the spectrum. A decrease in intensity and broadening of the extinction spectrum is observed with even further increase of the tilt angle, due to dephasing.

The present invention recognizes that the two (2) maximum peaks at 431 nm and 471 nm, are actually two (2) independent maxima whose contributions to the overall extinction spectrum vary with the tilt angle of the CMF to the light source. The shoulder at 431 nm, which remains high as the tilt angle increases, supports this conclusion. However, this may alternatively be thought of as a gradual evolution of a single maximum and 431 nm and 471 nm maxima are its extreme positions.

At a normal incidence angle of 0°, multipolar oscillations are in a plane parallel to the surface of the CMF. When tilted, the component normal to the surface becomes excited. Because the components for different particles are parallel to each other, the particles will couple as dipoles rather than quadrupoles. The 471 nm peak is attributed to a blue-shifted, from the red spectral region at about 520 nm, dipole plasmon oscillation of single particles. The blue shift occurs as a result of exciton-like interactions similar to those in molecular H-aggregates. Thus, the present invention recognizes that the complex behavior of the high density CMF extinction spectra results from the coupling of different harmonics of the plasmon resonances due to changes in the tilt angle of the CMF relative to the p-polarized light source.

One possible application of the present invention is utilizing the invented colloidal metal films as an "artificial antenna". The nanometer sized silver particles of the preferred embodiment of the present invention possess

highly efficient light harvesting properties and have extreme stability to high doses of radiation and relative simplicity of preparation. The strong coupling of plasmon resonances in the present invention allows light energy adsorbed by one particle to be transferred through the particle's array at some distance. Thus, the CMFs of the present invention may be used as a highly efficient antenna in different types of devices utilizing the energy of light.

Another possible application of the present invention is a "trap" assay, wherein the collective resonance may be efficiently disturbed by molecular chromophores with a high extinction coefficient in the spectral region of the collective plasmon resonance, resulting in adsorption of energy by the chromophore. The chromophore can then be used as a label in a competitive immunoassay to estimate the amount of labeled molecules bound to the CMF. This approach should be substantially sensitive, since only a few chromophores are necessary to produce considerable changes in the CMF's extinction spectrum.

Another potential application of the present invention is for measurements of dielectric constant and detection of small amounts of impurities. The position and the intensity of the collective resonances are sensitive to dielectric constant of their surrounding medium and organic and inorganic molecules on the silver particle's surface also influences the spectral properties of the colloidal films. The different molecules have different physicochemical properties causing distinguishable changes in the CMF's UV-Vis spectral region. Thus, very small amounts, such as submonolayers, of molecules with high affinity to silver metal can be detected.

Additionally, the present invention may be applied to

perform surface-enhanced spectroscopies, such as Surface-Enhanced Fluorescence (SEF) and Surface-Enhanced Raman Spectroscopy (SERS). Colloidal metal films are potentially the most uniform and reproducible substrates for surface-enhanced spectroscopies. Because the films are very uniform, they can also be used for imaging of large biological objects, such as cells, utilizing both surface-enhanced Raman and surface-enhanced fluorescence microspectroscopies. The plasmon resonances of the films enhance optical signals, with the enhancement of the signal increasing as the surface density of metal particles on the film surface increases.

Because these colloidal films exhibit narrow and strong extinction bands in the visible region of the electromagnetic spectrum (from ca. 400-500 nm) they can be used as optical filters in this spectral region and prevent transmission of light at certain wavelengths. The transmission properties of the films are also dependent upon the angle of incidence of the light. Thus, they may be used to block different wavelengths of light by varying the angle of incidence.

The CMFs of the present invention may further be used in biomedical applications, such as homogenous immunoassays, analysis of whole blood, and DNA probes. The advantages of the CMFs of the present invention enable work on combining of polymerase chain reaction (PCR), which is used to selectively amplify DNA fragments, with detection of a DNA fragment of interest (DNA probe). The PCR and DNA probe are combined in a vial, with the present invention obviating the need for any intermediate steps, to greatly facilitate DNA probe techniques.

Another possible biomedical application of CMFs of the present invention, is a combination of the films with biological chips. The CMFs and biological chips can be used

for immunoassay and DNA probe applications, as well as additional medical diagnostic applications. A further potential field for the present invention, is the application of the present invention in biosensors based on fiber optics.

BRIEF DESCRIPTION OF THE DRAWINGS

The above and other features, organizations, advantages and objects of this invention will be fully understood from the following detailed description and the accompanying drawings. Each of the drawings contained herein are not considered to be accurate depictions of the embodiments of the invention, but are provided for illustrative purposes only and are to be interpreted in conjunction with the attached specification.

FIG. 1 is a graphical depiction of the extinction spectra of a low density colloidal metal film and a colloidal suspension used to produce the colloidal film of the present invention;

FIG. 2 is a graphical depiction of the extinction spectra of colloidal metal films of the preferred embodiment of the present invention;

FIGS. 3A-3D are electron micrographs of the colloidal metal films having different particle densities corresponding to the extinction spectra of FIG. 2; and

FIGS. 4A and 4B are graphical depictions of the extinction spectra of the colloidal metal film of the present invention showing the effects of s-polarized light, p-polarized light, and tilt angle of the film to the light, on the extinction spectrum of the colloidal metal film of the present invention.

FIG. 5 is a perspective depiction of the preferred embodiment of the present invention.

DETAILED DESCRIPTION OF THE PERFORMED EMBODIMENT OF THE INVENTION

The following description is provided to enable any person skilled in the art to make and use the invention, and sets forth the best modes presently contemplated by the inventors for carrying out this invention. Various modifications, however, will remain readily apparent to those skilled in these arts, since the generic principals of the present invention have been defined herein.

The present invention provides strong nonradiative coupling of plasmon resonances, which are collective oscillations of electron density in metals, of closely spaced, metal particles arranged in semi-regular, two-dimensional arrays. The metal particles of the preferred embodiment of the present invention comprise silver metal and are preferably nanometer sized. However, larger or smaller silver metal particles may also be utilized in the present invention. Additionally, other suitable nanometer-sized metal or semiconductor particles that also have appropriate physical properties in two-dimensional semi-regular arrays may be used within the scope of the present invention.

The preferred embodiment of the present invention introduces colloidal metal films (CMFs) for use as substrates for enhanced spectroscopies; optical filters; and active elements in energy-harvesting, transformation, and transfer systems. The colloidal metal films of the present invention are prepared by adsorption, such as by self-assembly, of metal particles on derivatized substrates.

In the preferred embodiment of the present invention,

the colloidal metal films are prepared on glass slides derivatized with 3-mercaptopropyl trimethoxy silane (MPS). However, the colloidal metal films can be formed on any solid substrate, that have functional groups with high affinity to silver, or other appropriate metal to be used. For silver, such groups include -SH, -CN, pyridyl, and $-NH_2$, for example.

In the preferred embodiment, the slides are exposed to a colloidal suspension of silver metal particles having average diameters in the range of 200-1000 Ångstroms for a period of time approximately twenty four (24) hours.. After exposure of the slides to the colloidal suspension, an irreversible adsorption of the metal to the surface results, due to the formation of silver-sulphur bonds.

Because of the electrostatic interaction between individual particles, a semi-regular two-dimensional array of the silver particles is produced. After exposure of the slides to colloidal suspensions for a substantially extended time period, such as several days for example, highly dense metallic films are formed, in which the mean distance between particles is approximately equal to, or less than, their average diameter.

Referring now to FIG. 1 and FIGS. 3A-3D of the drawings, there is shown the extinction spectrum 10 of the colloidal suspension used for the preparation of the colloidal metal films (CMFs) of the preferred embodiment of the present invention, together with the extinction spectrum 12 of a low particle density CMF, such as that shown in FIG. 3A, wherein the mean distance between particles 14 is significantly greater than their diameter. Because the interparticle spacing in the colloidal suspension of optical density 1.0 is approximately 5 μm, the extinction spectrum 10 thereof can be thought to represent the plasmon resonances of noninteracting single particles 14 (FIGS. 3A-3D).

It should be noted that in order to achieve interparticle spacing in colloidal suspensions comparable to that of a highly dense CMF, an optical density on the order of 10^4 is required. Practically speaking, this is impossible due to the aggregation of colloids at high concentrations.

Several distinct features are observed in each of the extinction spectrum 10, 12 of noninteracting particles 14. Firstly, a maxima 16 appears in each of the spectrum 10, 12 near 510 nanometers (nm), and another maxima 18 appears in each of the spectrum 10, 12 near 425 nm. Also, a first shoulder 20 appears in each of the spectrum 10, 12 at approximately 380 and a second shoulder 22 appears about 350 nm in each spectrum 10, 12. These features can be attributed to excitation of different harmonics of the plasmon resonance, for a nearly spherical shaped particle. The measured extinction spectra 10 of the suspension also reflects the size variance of the particles 14. As shown in FIGS. 3A-3D, the size variance of the particles is substantially small, i.e., each of the particles have generally the same diameter.

Referring now to FIG. 2 and FIGS. 3A-3D of the drawings, each of the spectra 24, 26, 28, and 30 of FIG. 2 corresponds the CMFs of FIGS. 3A, 3B, 3C and 3D, respectively. The spectra 24, 26, 28, and 30 were obtained at a normal incidence angle, wherein the CMF is at an angle of substantially zero degrees (0°) to light emitted toward the CMF. As can be seen from the extinction spectra 24, 26, 28, and 30, as the density of the particles 14 in the CMF increases, a dramatic change is observed in the spectra 24, 26, 28, and 30.

First, a low density CMF at 1.4 particles/μm^2 as shown in FIG. 3A, has an extinction spectrum 24 with a somewhat broad

and low intensity maxima 32. A more dense CMF at 14 particles/μm^2 as shown in FIG. 3B, has an extinction spectrum 26 with a maxima 34 that is narrower and more intense than the maxima 32 of the low density CMF. The maxima 34 of the denser CMF also shifts slightly to the blue spectral region. The density CMF at 20 particles/μm^2 of FIG. 3C has an extinction spectrum 28, wherein the lowest energy maximum of the spectrum 28 shifts further to the blue spectral region and collapses into a single peak 36 at approximately 435 nm.

The high density CMF at 25 particles/μm^2 of FIG. 3D has an extinction spectrum 30, wherein the lowest energy maximum spectrum 30 appears to shift to the blue spectral region and finally, collapses into a single sharp peak 38 at approximately 431 nm. This is an "enhanced" peak, which corresponds to silver particles 14 coupled through quadrupole resonances. The enhanced peak 38 occurs when the mean distance between particles 14 is less than or equal to their diameter.

Light can excite dipolar and multipolar harmonics of the plasmon resonances in the single particles. These excited harmonics will be coupled with harmonics of the nearby particles. The preferred embodiment of the present invention recognizes that simple symmetry considerations from having a two-dimensional arrangement of the metal particles can result in excitation of multipolar harmonics. This conclusion can be supported by the fact that the sharp enhanced peak in the extinction spectrum is more intense in CMFs with large silver particles, which maintain an interparticle distance equivalent to their diameters.

Referring now to FIGS. 4A and 4B of the drawings, there is shown the extinction spectrum 40 of a CMF exposed to S-polarized light (FIG. 4A), and the extinction spectrum 42 of

the CMF exposed to P-polarized light (FIG. 4B). The spectra 40, 42 were generated from a CMF that has interparticle spacing approximately equal to the average diameter of particles 14 therein. The spectra 40, 42 are dependant upon the tilt angle of the film from a normal incident angle of light exposed thereto, to be discussed hereafter. The fact that the spectra 40, 42 are dependent upon the tilt angle is evidence of collective plasmon resonances of the particles 14 in the CMF. With low particle density CMFs, there is no unusual angular dependence of their extinction spectra (not shown).

Referring to FIG. 4A, when s-polarized light is applied to the CMF, the extinction spectrum 40 broadens and decreases in intensity as the tilt angle of the CMF relative to the light is increased. The spectrum 40 broadens and decreases in intensity due to dephasing of the plasmon oscillation in neighboring silver particles 14. Dephasing is due the incident wavefront of the light reaching the silver particles in the colloidal film at different phases, as a result of the tilt angle increasing from a normal incident angle of zero degrees (0°) relative to the light, to a substantial angle.

Referring to FIG. 4B, when the CMF is exposed to p-polarized light, the dependence of the extinction spectrum 42 is more complex. As the tilt angle of the CMF is increased from the normal incident angle of 0°, an enhanced sharp peak or main maximum 44 in the spectrum, at about 431 nm, first decreases in intensity, slightly broadens around 450 nm, then shifts to the red spectral region. The plasmon resonance reaches a minimum 48 in intensity at around a 30° angle of the film relative to the light.

The maximum 44 continues to shift to the red spectral region, increases in intensity, and reaches another maximum

46 around 55° of the film to the light at the 471 nm position of the spectrum 42. A decrease in intensity and broadening of the spectrum 42 is observed with even further increase of the tilt angle, due to dephasing.

It appears that the two (2) maximum peaks at 431 nm and 471 nm, are actually two (2) independent maxima 44, 46, contributions of which to the overall extinction spectrum 42 vary with the tilt angle of the CMF to the light. This conclusion is supported by shoulder 48 in the spectrum 42 formed at about 431 nm, which remains high as the tilt angle increases. However, this may alternatively be thought of as a gradual evolution of a single maximum and 431 nm and 471 nm maxima 44, 46 are its extreme positions.

At a normal incidence angle of 0°, multipolar oscillations are in a plane parallel to the surface of the CMF. When tilted, the component normal to the surface becomes excited. Because the components for different particles 14 are parallel to each other, the particles 14 will couple as dipoles rather than quadrupoles. The maxima 46 at 471 nm is attributed to a blue-shifted dipole plasmon oscillation of single particles 14 from the red spectral region at about 520 nm. The blue shift occurs as a result of exciton-like interactions similar to those in molecular H-aggregates. Thus, the complex behavior of the high intensity CMF extinction spectra 42 results from the coupling of different harmonics of the plasmon resonances, due to changes caused by the tilt angle of the CMF to the p-polarized light source.

Therefore, the preferred embodiment of the present invention provides strong nonradiative coupling of plasmon resonances in semi-regular arrays of nanometer-sized silver metal particles. The present invention provides a new

approach to energy transfer and storage systems that has fundamental and practical importance in various appropriate applications.

FIG. 5 shows a perspective depiction of the preferred embodiment of the present invention. In FIG. 5, silver particles 14 are spaced in its semi-regular two-dimensional array upon a glass size substrate 50. The silver metal particles have a general nanometer diameter and spacial distance between each particle and its neighboring particle is depicted by the arrows S. Light L is infinite upon the colloidal metal film made up by the semi-regular two-dimensional array of silver metal particles 14 upon the slight 50 at an angle alpha.

One possible application of the present invention is utilizing the invented colloidal metal films as an "artificial antenna". The nanometer sized silver particles possess highly efficient light harvesting properties, even more efficient than the best natural molecular chromophores from green plants. The particles also have extreme stability to high doses of radiation and relative simplicity of preparation. The strong coupling of plasmon resonances in semi-regular arrays of silver particles in the CMFs of the present invention implies that light energy adsorbed by one particle may be transferred through the particle's array at some distance. The energy can then be adsorbed by some acceptors and used in further physicochemical reactions. Thus, the CMFs of the present invention may be used as a highly efficient antenna in different types of devices utilizing the energy of light.

The present invention may also be utilized in a "trap" assay, wherein the collective resonance may be efficiently disturbed by molecular chromophores with a high extinction

coefficient in the spectral region of the collective plasmon resonance. The disturbance of the resonance will occur as a result of adsorption of energy by the molecular chromophore. Such a chromophore can be used as a label in a competitive immunoassay to estimate the amount of labeled molecules bound to the CMF, with immobilized specific antibodies, by monitoring any decrease of the film's resonance.

This approach will be substantially sensitive, since only a few chromophores are necessary to produce considerable changes in the CMF's extinction spectrum. Further, nonspecifically bound labeled molecules, that are bound to glass or another support of the colloidal metallic film should not have any effect on the collective resonance, thus eliminating background problems.

Another potential application of the present invention is for measurements of dielectric constant and detection of small amounts of impurities. The position and the intensity of the collective resonances are sensitive to dielectric constant of their surrounding medium. Adsorption of small amounts of organic and inorganic molecules on the silver particle's surface also influences the spectral properties of the colloidal metal films. Since different molecules have different physicochemical properties, the molecules can cause distinguishable changes in the CMF's UV-Vis spectral region. Thus, very small amounts, such as submonolayers, of molecules with high affinity to silver metal can be detected this way.

Additionally, the present invention may be used to perform surface-enhanced spectroscopies, such as Surface-Enhanced Fluorescence (SEF) and Surface-Enhanced Raman Spectroscopy (SERS). The invented colloidal metal films are potentially the most uniform and reproducible substrates for surface-enhanced spectroscopies. Because the films are very

uniform, they can also be used for imaging of large biological objects, such as cells, utilizing both surface-enhanced Raman and surface-enhanced fluorescence microspectroscopies. The plasmon resonances of the films enhance optical signals, with the enhancement of the signal increasing as the surface density of metal particles on the film surface increases. Therefore, preparation of colloidal metal films which possess collective resonances is also a way to prepare highly reproducible and uniform substrates for surface-enhanced spectroscopies.

In one application of the preferred embodiment of the present invention, Surface-Enhanced Fluorescence (SEF) Spectroscopies are performed using an avidin/biotin-FITC system. Because these colloidal films exhibit narrow and strong extinction bands in the visible region of the electromagnetic spectrum (from ca. 400-500 nm) they can be used as optical filters in this spectral region and prevent transmission of light at certain wavelengths. The transmission properties of the films are also dependent upon the angle of incidence of the light. Thus, they may be used to block different wavelengths of light by varying the angle of incidence. In this embodiment, an enhancement of fluorescence up to 50 times has been observed for CMFs of the present invention. With further implementations of the invention even this value can be improved considerably.

Another implementation of the preferred embodiment of the present invention is in Surface-Enhanced Raman Spectroscopy (SERS). In order to observe a strong Raman signal on the CMF of the present invention, an additional step of CMF preparation aggregates colloidal particles on the film's surface. The observed signals are significantly greater than well known conventional SERS-active substrates: colloidal suspensions, metal island films, or electrodes.

The CMFs of the present invention may further be used in biomedical applications, such as homogenous immunoassays, analysis of whole blood, and DNA probes. The advantages of the preferred embodiment of the present invention have enabled work on combining of polymerase chain reaction (PCR), which is used to selectively amplify DNA fragments, with detection of a DNA fragment of interest (DNA probe). The PCR and DNA probe are combined in a vial, with the preferred embodiment of the present invention obviating the need for any intermediate steps, to greatly facilitate DNA probe techniques.

Another possible biomedical application of CMFs of the present invention, is a combination of the films with biological chips currently under development. The CMFs and developing biological chips can be used for immunoassay and DNA probe applications, as well as additional medical diagnostic applications. A further potential field for the present invention, is the application of the present invention in biosensors based on fiber optics.

Those skilled in the art will appreciate that various adaptations and modifications of the just described preferred embodiments can be used and configured without departing from the scope and spirit of the invention. Therefore, it is to be understood that, within the scope of the appended claims, the invention may be practiced other than as specifically described herein.

CLAIMS

1. A colloidal film comprising:

a substrate;

a multiplicity of particles adsorbed on the substrate; and

wherein the particles are adsorbed to the substrate such that an electrostatic interaction between the particles forms a semi-regular array of the particles.

2. The colloidal film of Claim 1 wherein the particles have plasmon resonances and the particles are spaced on the substrate such that an irradiating light can excite dipolar and multipolar collective plasmon resonances in the particles.

3. The colloidal film of Claim 1 wherein a mean distance between particles is approximately equal to an average diameter of the particles.

4. The colloidal film of Claim 3 wherein the particles have an average diameter in the range of 200-1000 Ångstroms.

5. The colloidal film of claim 1 wherein the particles comprise silver metal.

6. The colloidal film of claim 1 wherein the substrate has functional groups with a high affinity to the particles.

7. The colloidal film of claim 6 wherein the

functional groups are selected from the group consisting of -SH, -CN, pyridyl, and $-NH_2$.

8. The colloidal film of claim 6 wherein the substrate comprises a solid.

9. The colloidal film of claim 8 wherein the substrate comprises glass derivatized with 3-mercaptopropyl trimethoxy silane for irreversibly adsorbing the particles to the substrate.

10. A method of producing a colloidal metal film comprising the steps of:

providing a substrate having a high affinity to a predetermined metal;

providing a colloidal suspension of a multiplicity of particles, the particles being the predetermined metal; each of the particles having a same approximate diameter; and

exposing the substrate to the suspension for a time period for adsorbing the particles to the substrate such that an electrostatic interaction between the particles forms a semi-regular array of the particles on the substrate, the time period being sufficiently long so that the particles are spaced on the substrate such that an irradiating light can excite dipolar and multipolar collective plasmon resonances in the particles.

11. The method of Claim 10 wherein the particles have an average diameter of 200-1000 Ångstroms.

12. The method of Claim 11 wherein a mean

distance between particles is approximately equal to an average diameter of the particles.

13. The method of Claim 11 wherein a mean distance between particles is slightly less than an average diameter of the particles.

14. The method of claim 10 wherein the particles comprise silver.

15. The method of claim 10 comprising further steps of:

providing a glass substrate; and

derivatizing the glass substrate with 3-mercaptopropyl trimethoxy silane to form silver-sulphur bonds between the particles and the substrate when the suspension is exposed to the substrate to adsorb the particles to the substrate.

16. The method of claim 15 wherein the substrate has functional groups with a high affinity to silver, the functional groups selected from the group consisting of -SH, -CN, pyridyl, and $-NH_2$.

17. A colloidal metal film comprising:

a substrate having a high affinity to silver; and

a multiplicity of silver metal particles adsorbed on the substrate, the particles adsorbed to the substrate such that electrostatic interaction between the particles forms a semi-regular array of the particles, with a mean distance between particles approximately equal to an average diameter of the

particles, the particles spaced on the substrate such that when the film is irradiated with light, dipolar and multipolar plasmon resonances are excited.

FIG. 1

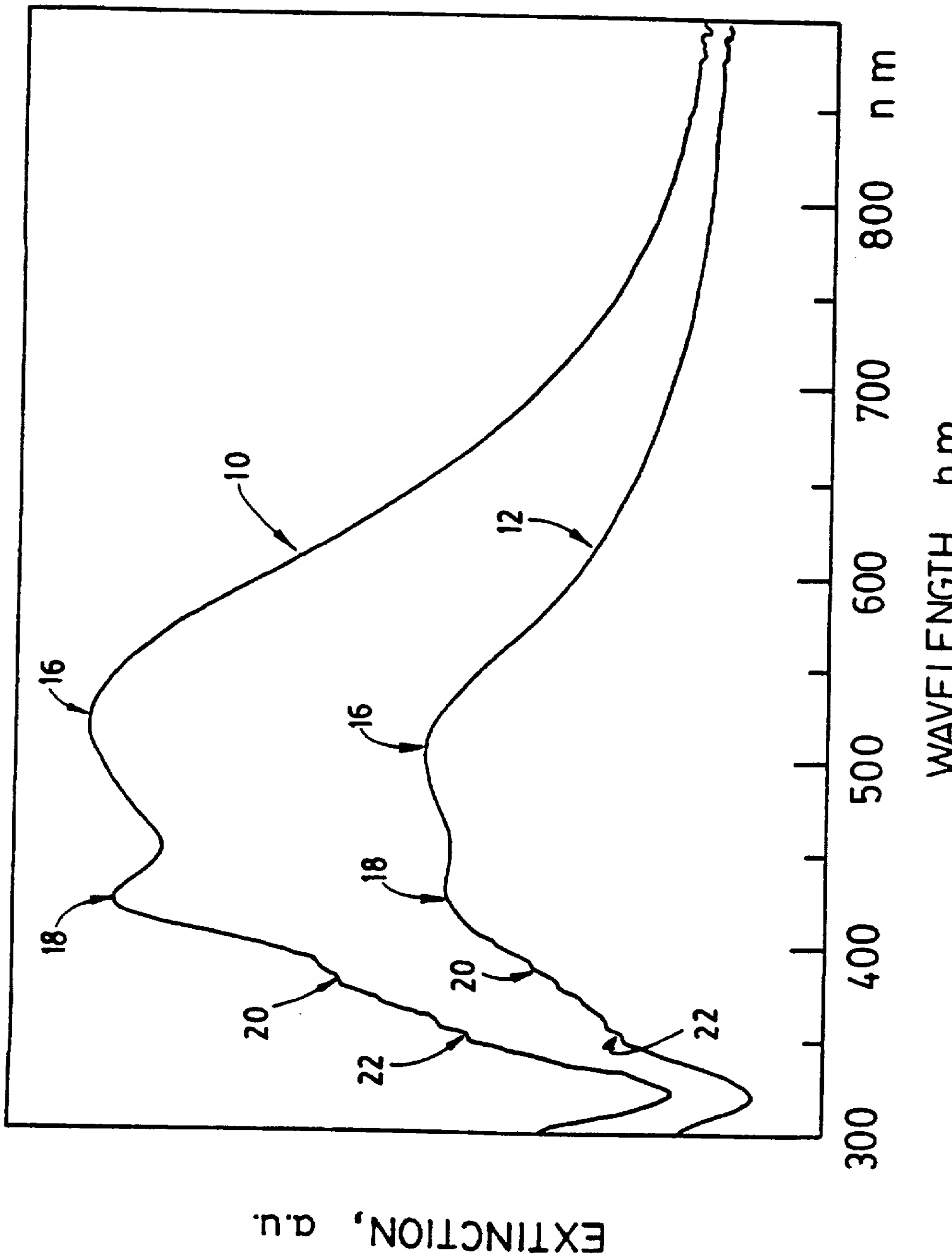
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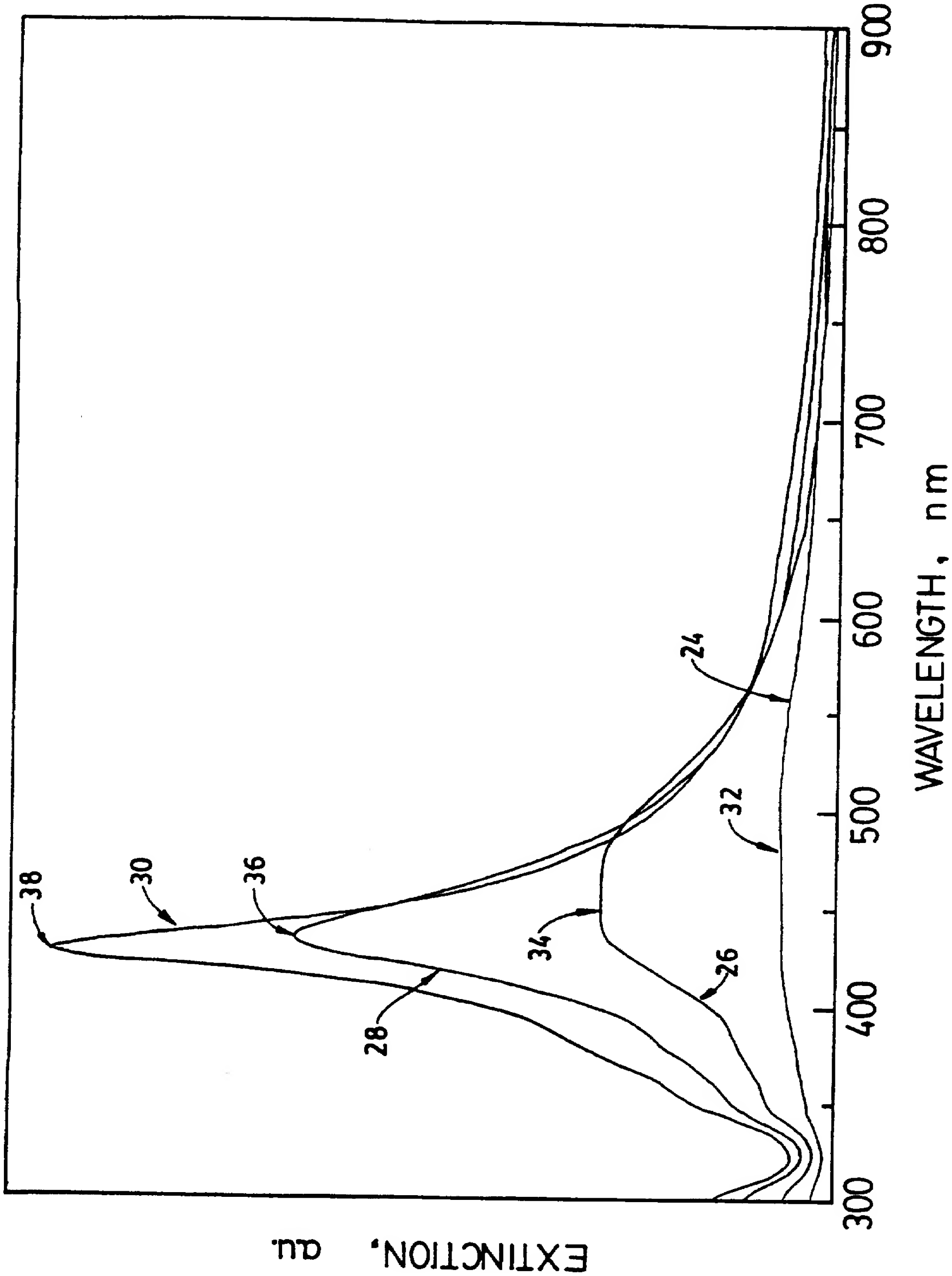
FIG. 2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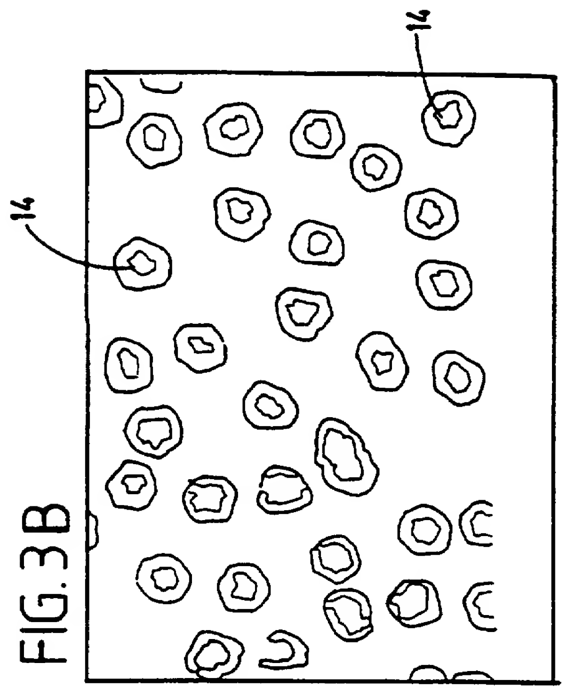

FIG. 3B

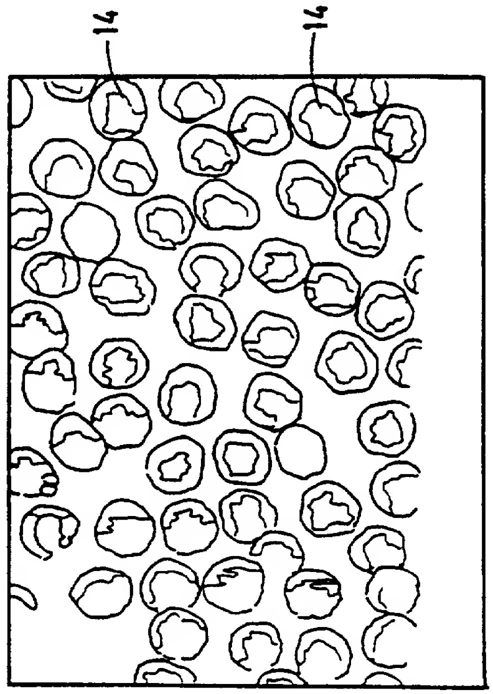

FIG. 3D

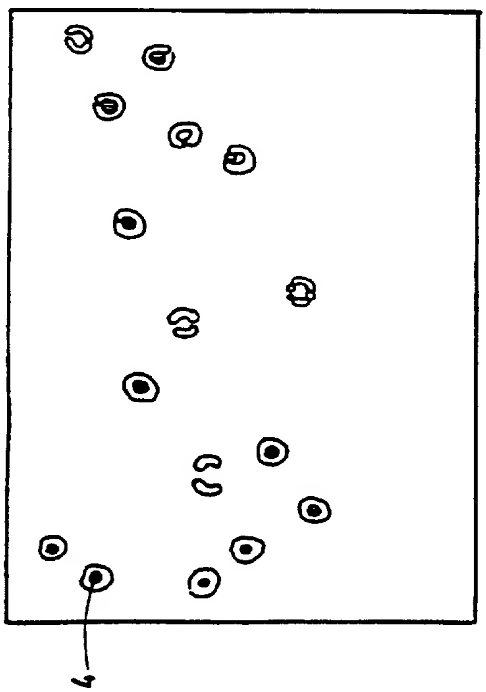

FIG. 3A

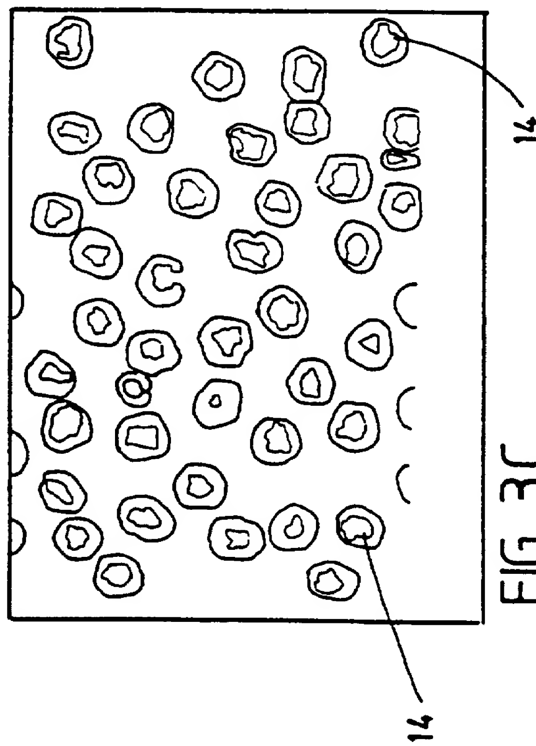

FIG. 3C

FIG. 4b

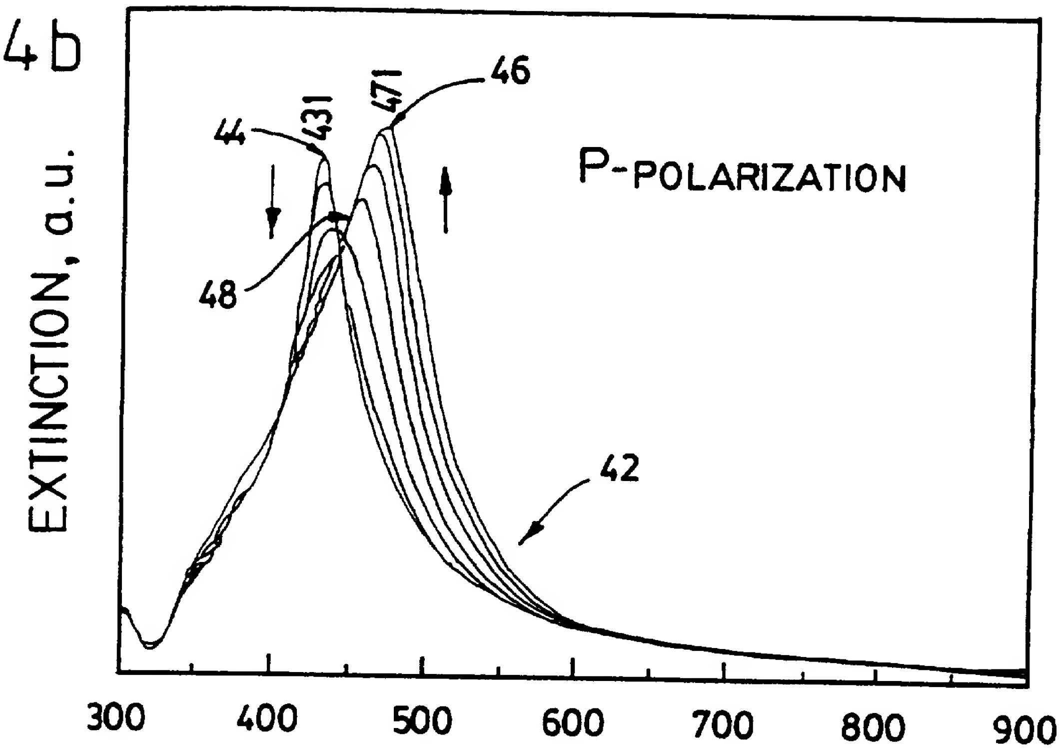
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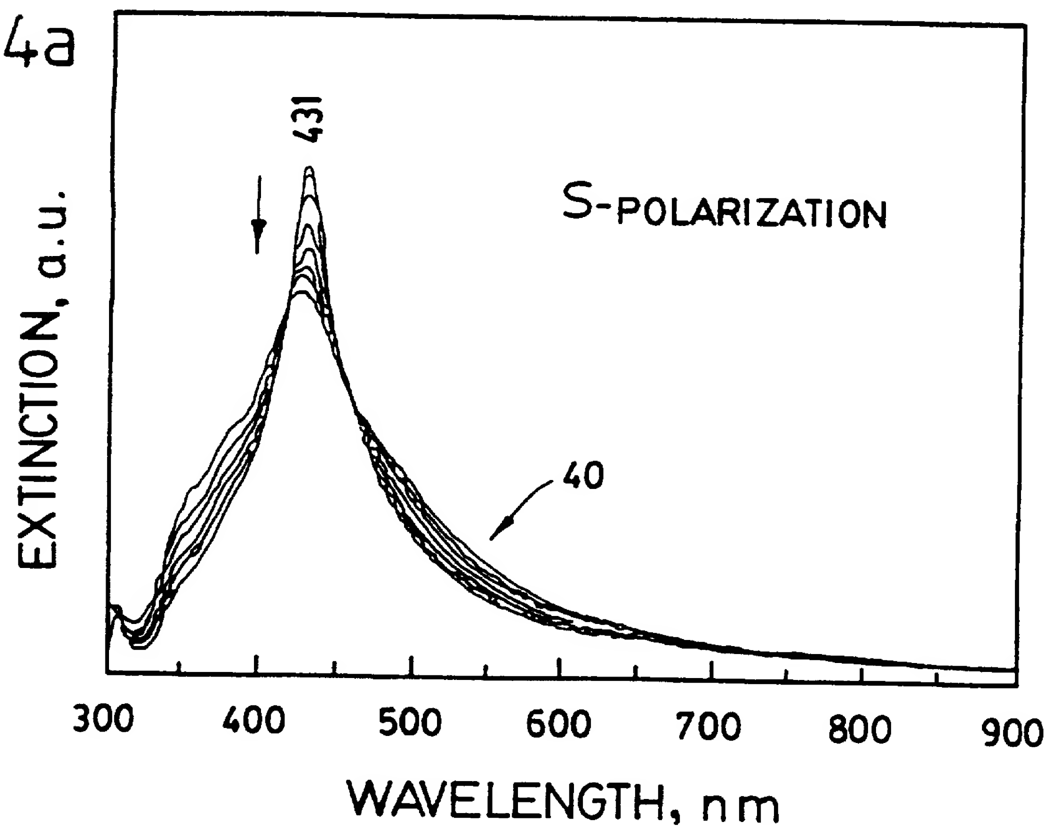
FIG. 4a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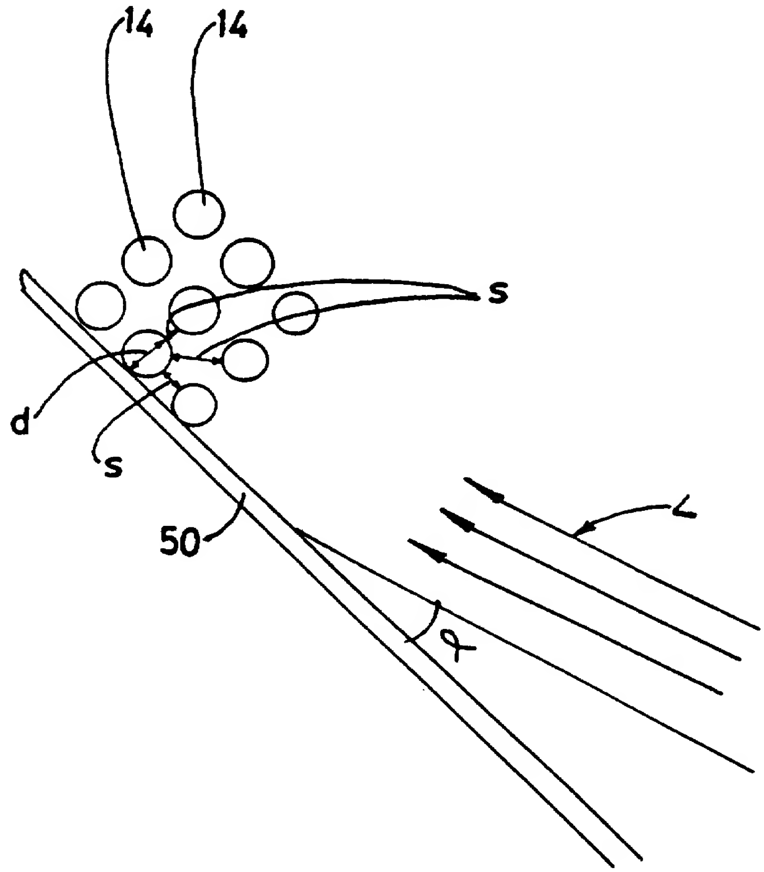
WAVELENGTH, nm

FIG. 5



INTERNATIONAL SEARCH REPORT

Intern. nal Application No
PCT/IB 97/01084

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N21/55

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHUMANOV, G.: "Colloidal metal films as a substrate for surface-enhanced spectroscopy" J. PHYS. CHEM, vol. 99, no. 23, 8 June 1995, pages 9455-9471, XP002049489 see page 9466 - page 9467; figure 1 ---	1-17

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
8 December 1997	09. 01. 98

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Mason, W

INTERNATIONAL SEARCH REPORT

Intern. .al Application No
PCT/IB 97/01084

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	FRITZSCHE W ET AL: "Ultrastructural characterization of colloidal metal films for bioanalytical applications by scanning force microscopy" 42ND NATIONAL SYMPOSIUM OF THE AMERICAN VACUUM SOCIETY, MINNEAPOLIS, MN, USA, 16-20 OCT. 1995, vol. 14, no. 3, pt.2, ISSN 0734-2101, JOURNAL OF VACUUM SCIENCE & TECHNOLOGY A (VACUUM, SURFACES, AND FILMS), MAY-JUNE 1996, AIP FOR AMERICAN VACUUM SOC, USA, pages 1766-1769, XP002049490 see page 1766 - page 1767; figure 1 ---	1-17
A	MORGAN, S. ET AL: "Reduction of Cytochrome c by Halide-Modified, Laser-Ablated silver colloids." J. PHYS. CHEM., vol. 100, no. 11, 14 March 1996, pages 4672-4678, XP002049491 see page 4672 - page 4673; figure 1 ---	1-17
A	WO 91 06894 A (RES CORP TECHNOLOGIES INC) 16 May 1991 see page 12 - page 25; claims 1,2; figures 1-13 -----	1-17

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern. nal Application No
PCT/IB 97/01084

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9106894 A	16-05-91	NONE	